Journal of Integrative Neuroscience

J. Integr. Neurosci. **2023**; 22(1): 14
https://doi.org/10.31083/j.jin2201014

Original Research

The Role of eIF5A1 in LPS-Induced Neuronal Remodeling of the Nucleus Accumbens in the Depression

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Academic Editor: Gernot Riedel
Submitted: 12 May 2022 Revised: 9 June 2022 Accepted: 18 July 2022 Published: 11 January 2023

Abstract

Background: The pathogenesis of depression is complex, with the brain's reward system likely to play an important role. The nucleus accumbens (NAc) is a key region in the brain that integrates reward signals. Lipopolysaccharides (LPS) can induce depressive-like behaviors and enhance neuroplasticity in NAc, but the underlying mechanism is still unknown. We previously found that eukaryotic translation initiation factor A1 (eIF5A1) acts as a ribosome-binding protein to regulate protein translation and to promote neuroplasticity. **Methods**: In the present study, LPS was administered intraperitoneally to rats and the expression and cellular location of eIF5A1 was then investigated by RT-PCR, Western blotting and immunofluorescence. Subsequently, a neuron-specific lentivirus was used to regulate eIF5A1 expression *in vivo* and *in vitro*. Neuroplasticity was then examined by Golgi staining and by measurement of neuronal processes. Finally, proteomic analysis was used to identify proteins regulated by eIF5A1. **Results**: The results showed that eIF5A1 expression was significantly increased in the NAc neurons of LPS rats. Following the knockdown of eIF5A1 in NAc neurons, the LPS-induced increases in neuronal arbors and spine density were significantly attenuated. Depression-like behaviors were also reduced. Neurite outgrowth of NAc neurons *in vitro* also increased or decreased in parallel with the increase or decrease in eIF5A1 expression, respectively. The proteomic results showed that eIF5A1 regulates the expression of many neuroplasticity-related proteins in neurons. **Conclusions**: These results confirm that eIF5A1 is involved in LPS-induced depression-like behavior by increasing neuroplasticity in the NAc. Our study also suggests the brain's reward system may play an important role in the pathogenesis of depression.

Keywords: depression; nucleus accumbens; eukaryotic translation initiation factor A1 (eIF5A1)

1. Introduction

Depression endangers the physical and mental health of individuals and affects their quality of life. According to WHO statistics, there are currently about 350 million people in the world with depression. This condition is expected to become the number one disease in humans by 2030 [1]. Lipopolysaccharide (LPS) activates CD14 and TLR4 signaling pathways to initiate inflammation-related responses [2]. LPS in the brain not only causes a wide range of acute inflammatory responses, but also leads to depressive-like behaviors in experimental animals. As a consequence, LPS has been widely used in depression-related research [3,4].

The pathophysiological mechanism of depression is unclear, but it is currently thought to result from the interaction of genetic factors with environmental factors. Multiple brain regions may be involved in depressive symptoms. For example, the neocortex and hippocampus contribute to cognitive memory functions that mediate symptoms such as memory impairment, feelings of worthlessness, hopelessness, self-blame, and suicide in patients with depression. Moreover, the striatum, nucleus accumbens (NAc) and amygdala are involved in emotional memory and regulate symptoms such as anhedonia, anxiety and decreased motivation. The hypothalamus regulates autonomic nervous system function and is associated with changes in sleep, appetite, energy and libido during depression [5,6]. In addition, studies have found that depression reduces synapses and causes atrophy and the reduction of neuronal volume in the hippocampus, frontal cortex and ventral striatum [7]. Autopsy studies of patients with depression have observed decreased neuronal volume in the pre-orbital cortex, a decreased number and volume of glial cells in the frontal cortex and pre-orbital cortex, decreased cortical thickness, and atrophy of the basal ganglia [8]. Morphological studies have confirmed that depression leads to a decrease in the number of neural spines in brain regions such as the prefrontal cortex, hippocampus and cortex [3,4,9]. Of note, spine density in the NAc was consistently found to be significantly elevated. For example, Ma *et al*. [4] found that neuronal spine density in the prefrontal cortex and hippocampus of LPS-induced depressed rats was decreased,

whereas it was significantly increased in the NAc. These changes were reversed after combined treatment with brexpiprazole and fluoxetine. Similar results were also found in antidepressant studies of TrkB. The number of spines in the NAc is increased in depressive conditions, whereas in other brain regions it is reduced [9]. Therefore, in the present study the aim was to explore the underlying mechanisms of spine density changes in the NAc.

The NAc includes an outer shell and an inner core and is an important brain region in the mesolimbic dopamine system [10]. This system is well known for its role in regulating the reward mechanism of the brain. Its regulation of feeding, mating and drug addiction is considered to be a possible factor in the development of depressive-like symptoms. More than 95% of the neurons in the NAc are GABAergic medium spiny neurons. The NAc can receive projections from the ventral tegmental area, the prefrontal cortex, and the amygdala. It therefore plays a pivotal role in many conscious actions. The symptoms of anhedonia, loss of interest, and lack of motivation in depressive disorder may be related to the NAc [11]. For example, there is evidence that ΔFosB in NAc plays an important role in depression and chronic stress resistance [12]. Therefore, we believe that abnormal changes in NAc neurons may be involved in depression-related mechanisms.

eIF5A1 is a translational regulator that has been shown to promote neuronal growth [13,14]. We previously reported that eIF5A1 can increase spinal cord neuroplasticity, promote neurofilament protein expression, and increase the formation of neuromuscular junctions [14,15]. eIF5A1 is specifically expressed in embryos and especially in differentiated and proliferating cells. Its expression is greatly reduced at the end of growth and development [16]. A high level of eIF5A1 expression in the developing brain has been reported [17] and this may increase neuronal growth and survival [13]. Mechanistic studies have shown that eIF5A1 is related to the cytoskeleton. The eIF5A1-specific inhibitor GC7 (CAS 150333-69-0) can block cell transition at the G1-S phase [18]. The mechanism by which eIF5A1 regulates ribosomal translation remains controversial. Blaha *et al.* [19] reported that the prokaryotic homolog of eIF5A1, EF-P, acts at the 30S and 50S junction of the ribosome, between the P and E sites. There is also evidence that eIF5A1 regulates the translation of consecutive proline structures on the peptide chain, e.g., PPP, PPG, etc. Such structures can slow down or even stop the translation process. eIF5A1 helps to release the consecutive prolines that form a peptide bond, thus accelerating the translation process [20,21]. The aim of this study was therefore to explore whether increased neuroplasticity of the NAc observed in depression is related to eIF5A1.

2. Methods

2.1 Animal

Adult male rats (weight 280–300 g) were used in this study. All animal experiments were approved by the Animal Research Committee of Chongqing Medical University and feeding and care followed the NIH Laboratory Animal Welfare Guidelines. There was a 12 h light/dark alternation mode, free access to water and food, and a room temperature of 25 °C ± 1.0 °C. LPS (L-4130, serotype 0111:B4, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in normal saline. Rats were administered intraperitoneally at a dose of 0.5 mg/kg.

2.2 Behavioral Analysis

2.2.1 Sucrose Preference Test (SPT)

The SPT test was performed on rats after LPS injection. The first two days were the adaptation stage, where two bottles of drinking water were placed in each cage and the position of the bottles was changed at 7:00 AM. From the third day, one bottle was filled with drinking water and the other with 1% sucrose water. Both were weighed before the test. The position of the bottles was changed every day at 7:00 AM to prevent the rats from favoring a certain bottle due to its location. The bottles were weighed after the fifth day and the sucrose preference was calculated as: sucrose water consumption/(sucrose water consumption + normal water consumption).

2.2.2 Tail Suspension Test (TST)

Rats try to escape after hanging from their tails but are unable to, whereupon they give up struggling and enter a special depressed immobility state. The degree of depression was estimated according to the time the rats were still. The rear 1/3 of the tail was fixed with tape and hung on a bracket, with the head about 15 cm from the ground. The first 2 min was the adaptation time. The immobility time from 3 to 6 min was recorded and analyzed using the small animal behavior tracking and recording software Smart 3.0 (Bioseb, Pinellas Park, FL, USA) .

2.2.3 Forced Swimming Test (FST)

The rats were placed in a transparent drum with a 12 cm diameter and which contained water to a height of 10 cm and at a temperature of about 23 °C. The rats try to escape at first, but give up the struggle a short while later. Similar to the TST, the first 2 min was the adaptation time and the quiescent time from 3 to 6 min was recorded and analyzed using Smart 3.0.

2.2.4 Lentivirus

The hSyn promoter was used to express eIF5A1 mRNA or shRNA so that the virus was specifically expressed in neurons. 293T cells were cultured in six-well plates. Once the cell density reached 80%, the medium was

replaced with opti-MEM without penicillin, streptomycin or serum. After incubation at room temperature for 25 min, 30 μL of Opti-MEM containing 0.5 μg expression plasmid, 1 μL packaging plasmid and 3 μL transfection reagent mixture was added to the 6-well plate and the cells further cultured at 37 °C for 72 h. Finally, the cell supernatant was collected and centrifuged at 500 g for 10 min at 4 °C. The supernatant was then filtered through a 0.45 μm filter and virus particles were enriched using a Virus Concentration Kit (C2901M, Beyotime, Shanghai, China).

2.2.5 Western Blotting

The NAc was lysed by RIPA, centrifuged at 12,000 g for 15 min, and the supernatant collected. The protein concentration was then measured by BCA assay. After protein denaturation, SDS-PAGE electrophoresis was performed and the proteins transferred to PVDF membranes and blocked with 5% milk. Primary antibodies were incubated as follows: eIF5A1 (1:5000, Abcam, Waltham, MA, USA), ACTB (1:2000, GeneTex, Irvine, CA, USA). Secondary antibodies (GeneTex, USA) were then incubated at 1:5000 dilution. The HRP-ECL luminescence method was used to detect signal.

2.2.6 RT-PCR

Total RNA was extracted using Trizol reagent (Thermo, USA) and the RNA concentration measured using NanoDrop (Thermo, Waltham, MA, USA). A reverse transcription kit (Vazyme, Nanjing, Jiangsu, China) was used for reverse transcription of 1 μg RNA. Amplification was then performed using SYBR Green PCR mix (Vazyme, China). Primers for ACTB and eIF5A1 were as described in our previous publications [15]. Finally, the relative expression level was calculated by the Delta-Delta-Ct (ddCt) method.

2.2.7 Immunofluorescence

After the rats were anesthetized with isoflurane, the heart was exposed and perfused with pre-cooled PBS to wash away blood in the blood vessels. Perfusion and fixation was then continued using 4% paraformaldehyde. The brain tissue was subsequently dehydrated in 4% paraformaldehyde solution containing graded 10%, 20% and 30% sucrose. The brain was sectioned coronally to the NAc and the sections blocked with 10% goat serum at 37 °C for 30 minutes. Primary antibodies were incubated overnight at 4 °C as follows: eIF5A1 (Abcam, USA, 1:400), Neun (Abcam, USA, 1:500). Sections were then incubated with fluorescently-labeled secondary antibodies (Abcam, USA, 1:1000) at 37 °C for 2 hours. Finally, the nuclei were stained with DAPI and the sections observed by fluorescence microscopy.

2.2.8 Golgi Staining

The staining was performed according to the manufacturer's protocol for FD Rapid Golgi Staining (FD Neurotechnologies). Under deep anesthesia with 60 mg/kg ketamine and 0.25 mg/kg medetomidine (i.p.), the rat brains were removed from the skull as quickly as possible and rinsed quickly in Milli-Q water to remove blood from the surface. Tissue preparation was performed according to the manual's description. Coronal sections of 200 μM thickness were cut on a freezing microtome and then stained using solutions C, D, E and ethanol, respectively. For analysis, 5 sections of NAc area were selected for each brain and 5 evenly distributed pictures were taken on each side of the NAc. As described previously [22,23], Neuron J and Sholl plugins of ImageJ software (http://imagej.nih.gov/ij/) was used to analyze neuroplasticity. The neuronal processes (axons and dendrites) of each neuron were traced using NeuronJ, a semiautomated tracing software. To describe the dendritic arborization in detail, Sholl analysis of neuronal arbors was used. This creates a series of concentric shells (circles) around the focus of a neuronal arbor and then counts how many times the arbor intersects the sampling shells. Sholl analysis was performed on reconstructed neurons using the Sholl Analysis Plugin for ImageJ 1.8.0 (NIH, Bethesda, USA).

2.2.9 Neuron Culture

One-day old neonatal rats were sacrificed by decapitation and the NAc was harvested and digested with papain for 20 min. After centrifugation, the pellet was resuspended in DMEM. The cells were seeded in dishes at a density of 2–5 $\times$ 10^5 cells/ml and cultured for 4 h. The medium was then changed to neurobasal containing B27 (Thermo, USA). When the cells reached 70% density, 10 μL of virus was added and then after five days the cells were imaged for analysis.

2.2.10 Proteomic Analysis

NAc neurons in which eIF5A1 expression was regulated by the virus were collected and lysed by RIPA. Peptides from different samples were labeled with isotope iTRAQ reagent and the samples analyzed by LC-ESI-MS/MS. Proteins were identified and analyzed using Mascot 2.3.02 (Matrixscience, USA). The differentially expressed proteins with a $>$2 fold-change and with $p < 0.05$ were screened for functional analysis. Considering the positive regulatory effect of eIF5A1 on protein translation, the up-regulated proteins were analyzed in the eIF5A1 overexpression ORF group, while down-regulated proteins were analyzed in the eIF5A1 low expression SH group. GO analysis was performed using DAVID online software https://david.ncifcrf.gov/.

2.3 Statistical Analysis

SPSS 20.0 software (IBM, USA) was used for statistical analysis, with the results expressed as mean ± standard deviation. The *t*-test was used for comparison between two groups, and one-way analysis of variance was used for comparison of three or more groups. A p value of <0.05 indicated a significant difference.

3. Results

3.1 LPS Causes eIF5A1 Up-Regulation in the NAc

LPS induces depression-like behavior in animals and is therefore widely used in depression research [3,4]. Several studies have shown neuroplastic changes such as spine density reduction in most areas of the brain after LPS administration. However, spine density in the NAc showed a significant increase after LPS [3,4,9]. We previously demonstrated that eIF5A1 acts as a translational regulator to increase neuroplasticity [14]. In the present study, rats received a single intraperitoneal injection of LPS (0.5 mg/kg) and the expression of eIF5A1 in the NAc was subsequently evaluated. Fig. 1A shows increased protein and mRNA levels for eIF5A1 after LPS administration. Immunofluorescence was used to localize eIF5A1 expression in the NAc. Fig. 1B shows that eIF5A1 co-localized with the neuronal marker NeuN, indicating that it was expressed in neurons. Consistent with the results of molecular experiments, immunofluorescence showed that eIF5A1 expression in neurons was significantly increased after LPS administration. These results suggest that eIF5A1 may be involved in the LPS-induced increase in the density of NAc spines.

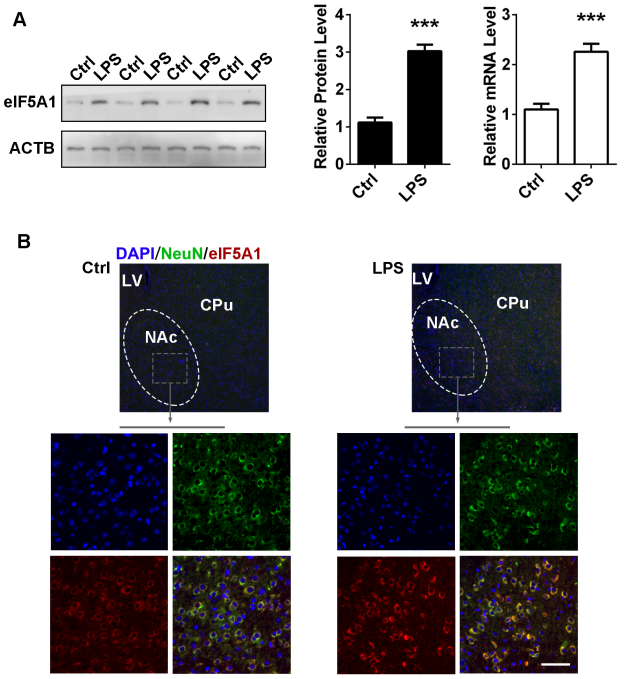

Fig. 1. eIF5A1 is up-regulated in the NAc of LPS rats. (A) Western blotting and PCR showed that LPS increased the expression of eIF5A1 (n = 8). (B) Immunofluorescence confirmed the expression of eIF5A1 in NAc neurons (n = 5). LV, lateral ventricle; CPu, caudate putamen; NAc, nucleus accumbens; NeuN, neuronal marker; bar = 50 μm. *** $p < 0.001$, compared with Ctrl group.

3.2 LPS Induces Neuroplasticity in the NAc

To confirm that LPS induces neuroplasticity in the NAc, rats brains were sectioned and the Golgi apparatus stained to observe neuronal morphological changes. NAc neurons were photographed systematically in different planes, thus allowing morphological maps of the entire neuron to be created. The neuronal processes (axons and dendrites) of each neuron were traced using NeuronJ, a semi-automated tracing software. Sholl analysis was used to describe the dendritic arborization in detail. This creates a series of concentric shells (circles) around the focus of a neuronal arbor and then counts how many times the arbor intersects the sampling shells. Sholl analysis was performed on reconstructed neurons using the Sholl Analysis Plugin for ImageJ. Fig. 2A shows a neuron in the NAc and its tracings. Sholl analysis was used to describe neuronal arbors. LPS treatment led to increased neuronal intersections and branches, as shown in Fig. 2B,C. The spine density was also increased in LPS rats, as shown in Fig. 2D,E. These results suggest that LPS induces neuroplasticity in the NAc.

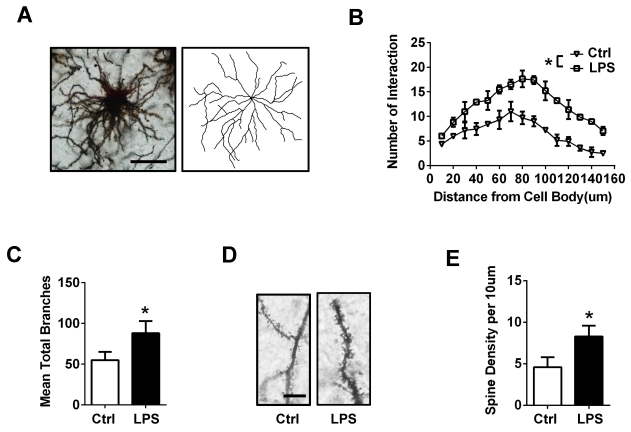

Fig. 2. LPS increases NAc neuroplasticity. (A) The schematic for neuronal Golgi staining and the tracing of neurites bar = 50 μm. (B) The intersection with shells was increased after LPS treatment. n = 50 neurons per group. (C) Mean total branches per group. LPS increased branches. n = 50 neurons per group. (D) Representative spine images for the two groups. bar = 10 μm. (E) Quantitative analysis of spine density. LPS increased spine density. n = 100 dendrites per group. * $p < 0.05$, compared with Ctrl group.

3.3 Knockdown of eIF5A1 Reduces LPS-Induced NAc Neuroplasticity

To confirm the role of eIF5A1 in LPS-induced NAc neuroplasticity, lentivirus was used to decrease eIF5A1 expression in rats NAc. The interfering RNA sequence for eIF5A1 was described in our previous study [24]. The hSyn promoter was used to regulate shRNA expression and thus to specifically knockdown eIF5A1 in neurons. Following injection of the virus into the NAc, molecular experimentation was subsequently performed to evaluate eIF5A1 expression. Western blotting results showed that eIF5A1 expression in the NAc of LPS-treated rats was significantly increased compared to the control group, whereas it was significantly reduced after virus injection. On the other hand, the control virus constructed using random sequences had no effect on the increased expression of eIF5A1 in the NAc (Fig. 3A). Reverse transcription PCR was used to measure mRNA expression and similar results to the protein changes were obtained (Fig. 3B). The neuroplasticity changes in NAc were also analyzed by Golgi staining. Sholl analysis revealed that LPS increased neuron intersections and branches (Fig. 3C,D). eIF5A1 knockdown in the NAc significantly decreased the intersection and branches of neurons, whereas the control virus had no effect on the LPS-induced increase in intersections and branches. Spine density also increased in LPS-conditioned rats, but was reduced in rats receiving intra-NAc infusion of Sh-eIF5A1 (Fig. 3E,F). These results suggest that eIF5A1 is involved in LPS-induced NAc neuroplasticity.

The NAc plays an important role in the brain's reward system, which has in turn been implicated in depressive behavior. Therefore, to further analyze whether eIF5A1 could regulate LPS-induced depression-like behaviors, we conducted the Sucrose Preference Test (SPT), Tail Suspension Test (TST) and Forced Swimming Test (FST) in a rat model. For the SPT, the consumption of sucrose water was significantly lower in LPS rats. And the resting time of the TST and FST was significantly longer in LPS rats (Fig. 3G–I). Together, these results suggest that LPS induces depression-like behavior in rats. After knockdown of eIF5A1 in the NAc, the consumption of sucrose water in LPS rats increased significantly, while the immobility time in the TST and FST was significantly shorter, indicating a reduction in depression-like behavior. The random sequence virus used as a control did not change the depression-like behavior in rats (Fig. 3G–I). This result suggests that LPS increases eIF5A1 expression in the NAc to promote neuroplasticity, and this change is involved in the depression-like behavior of rats.

To confirm the effect of eIF5A1 *in vitro*, NAc neurons from one-day old neonatal rats were isolated and cultured. The expression of eIF5A1 in neurons was subsequently regulated by lentivirus. Neurite outgrowth from these neurons was examined 5 days after lentivirus infection. Compared to the control group, the eIF5A1 up-regulated group showed a significant increase in neurite length, whereas the eIF5A1 down-regulated group showed a significant decrease in length (Fig. 4A,B). The control virus did not significantly alter the neuronal processes. Therefore, eIF5A1 was again confirmed to promote the plasticity of NAc neurons.

3.4 Proteome Analysis Confirms that eIF5A1 Regulates Neuroplasticity-Associated Proteins

eIF5A1 plays an important role in the translation of mRNA by acting as a ribosome-binding protein to regulate the translation of specific proteins. Deletion of eIF5A1 in cells results in decreased or even halted translation [20], indicating that it positively regulates mRNA translation. We performed iTRAQ proteomic analysis of *in vitro* lentivirus-infected NAc neurons in order to study the positive regulatory effect of eIF5A1 on protein translation. Proteins with elevated expression in the eIF5A1 up-regulated group, and those with decreased expression in the eIF5A1 down-regulated group were compared to the control group. A total of 227 proteins were identified, of which 129 had >2-fold changes with a p value < 0.05 (results are presented in the **Supplementary materials)**. Of these, 52 are closely associated with neuroplasticity (Fig. 5A). Functional analysis performed on the 129 differentially expressed proteins found that biological process (BP) was mainly enriched in items such as synaptic vesicle endocytosis, actin cytoskeleton organization and axon guidance. In the cellular component (CC), items such as axon, synapse and neuron projection are mainly enriched (Fig. 5B).

Next, we analyzed five proteins with important roles in neuroplasticity from the proteome results (Marked in red in Fig. 5A). Western blotting was used to measure their protein level and similar results to the eIF5A1 changes. Their expression in the NAc of LPS-treated rats was significantly increased compared to the control group, whereas it was significantly reduced after virus injection. On the other hand, the control virus constructed using random sequences had no effect on the increased expression of them in the NAc (Fig. 5C). These results confirm that eIF5A1 has a promoting role in LPS-induced neuroplasticity in the NAc.

4. Discussion

4.1 The Role of Inflammation in Depression

Depression is an important psychological disorder characterized by significant and persistent dark mood and aversion to activity as the main clinical features. Depressive episodes are often accompanied by slow thinking, decreased volitional activity, and other somatic symptoms such as loss of appetite and weight loss [3]. The involvement of neuroinflammation in the development of depression has been widely discussed and it is generally believed there are two important pathways. First, some peripheral cytokines can cross the blood-brain barrier (BBB) to cause an inflammatory response [25]. Second, there exists a "lo-

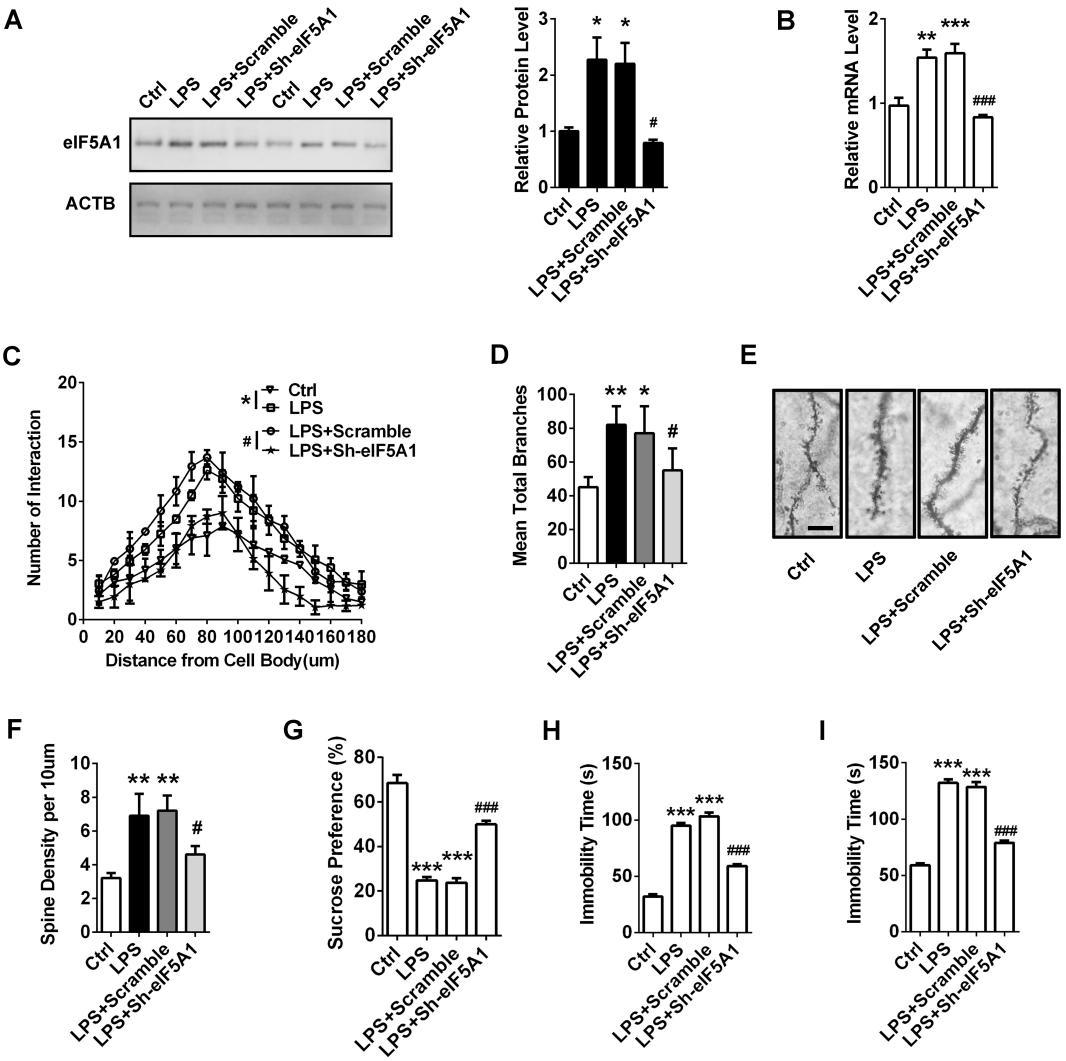

Fig. 3. eIF5A1 knockdown in the NAc inhibits LPS-induced neuroplasticity. (A,B) Western blotting and RT-PCR analysis showed that eIF5A1-shRNA lentivirus significantly decreased eIF5A1 expression in the NAc. n = 8 per group. (C) eIF5A1 knockdown (LPS + Sh-eIF5A1) significantly decreased the intersections. n = 50 neurons per group. (D) The mean total branches per group. LPS increased the number of branches, while eIF5A1 knockdown decreased the number of branches. n = 50 neurons per group. (E) Representative spine image for the three groups. bar = 10 μm. (F) Quantitative analysis of spine density. LPS increased spine density, while eIF5A1 knockdown reversed it. n = 100 dendrites per group. (G) The Sucrose Preference Test (SPT) results showed that the consumption of sugar water was significantly reduced in the LPS treatment group. eIF5A1 knockout increased sucrose water consumption. n = 8 per group. (H,I) The still time for the Tail Suspension Test (TST) and for the Forced Swimming Test (FST) increased after LPS treatment, whereas eIF5A1 knockdown reversed them. n = 8 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with Ctrl group; # $p < 0.05$, ### $p < 0.001$, compared with LPS + Scramble group.

cal immune system". For example, microglia are in a quiescent state under physiological conditions. However, when the brain is subjected to external stimuli such as trauma or infection, microglia turn into active forms and secrete a large number of pro-inflammatory cytokines, chemokines and reactive oxygen species [26]. Previous studies have shown that inflammation is related to depression. It has been reported for example that macrophage-induced inflammation plays a key role in the pathophysiology of depression [27]. In addition, patients suffering depression often have increased levels of pro-inflammatory factors such as TNF-α and IL-1β in their blood. The administration of pro-inflammatory factors such as LPS induces depression-like behaviors. Moreover, inflammation-related diseases such as arterial inflammatory disease and cerebral ischemia often cause depressive symptoms [28–30]. Therefore, LPS-induced depression-like behavior in a rat model can provide an effective research tool to study the mechanism of depression.

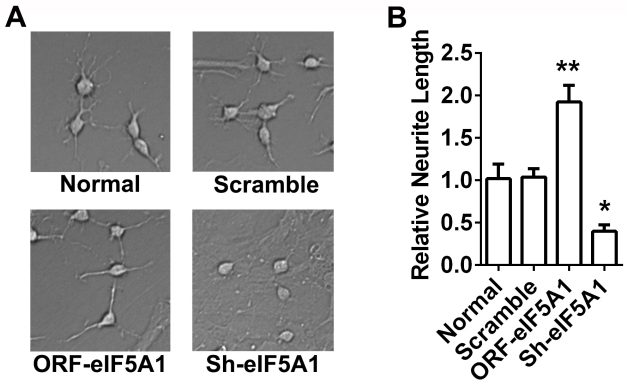

Fig. 4. eIF5A1 increases neurite outgrowth in NAc neurons. (A) Representative neuron images of eIF5A1 expression regulated by lentivirus. bar = 50 μm. ORF-eIF5A1, eIF5A1 up-regulation. Sh-eIF5A1, eIF5A1 down-regulation. (B) Statistical analysis of neurite outgrowth. n = 50 neurons per group. * $p < 0.05$, ** $p < 0.01$, compared with Ctrl group.

4.2 LPS may Induce Neuroplasticity by Altering the Microenvironment of the NAc

Increasing evidence suggests the pathogenic basis for depression involves the mesolimbic dopamine system. This system is well known for regulating the reward mechanism of the brain. Regulation of feeding, mating and drug addiction by the mesolimbic dopamine system is thought to be a factor in the development of depressive-like symptoms. The two most important brain regions in this system are the ventral tegmental area (VTA) and the nucleus accumbens (NAc) [11]. Dopaminergic neurons of the VTA send dopamine neurotransmitter to the GABAergic medium spiny neurons (MSNs) of the NAc. The NAc is composed of two parts, the core and the shell. GABAergic MSNs comprise more than 95% of all neurons in the NAc. Dopaminergic transmission that affects the VTA-NAc pathway can regulate depression-like behavior in animal models [31].

Our and other studies found that LPS could elevate the neuroplasticity in the NAc (Fig. 2) [4,32,33]. However, adding LPS directly in cultured neurons couldn't induce neuroplasticity, but neurotoxicity [34–36]. There are two possible mechanisms that we speculate. First is that neuronal projections from other brain regions alter NAc neuroplasticity. The NAc receives projections from glutamatergic neurons in the prefrontal cortex, hippocampus, amygdala and hypothalamus, as well as diffuse projections of norepinephrine and serotonin [37]. Moreover, the basolateral amygdala (BLA) also projects to the NAc. Suppressing the activity of BLA neurons can effectively alleviate depression-like behaviors [38]. The NAc is clearly a major hub that integrates signals for the brain reward system. Under LPS stimulation, co-projection from multiple brain regions may enhance neuroplasticity of the NAc. The second is that LPS improves NAc neuroplasticity by glial cells. Stimulating primary microglia and astrocytes with LPS increased neurotrophic factors secretion [39,40]. These neurotrophic factors may promote neuroplasticity in the NAc by increasing the branches and spines density of neurons. Therefore, LPS may not directly target neurons but alter the neuronal microenvironment of the NAc to induce neuroplasticity.

4.3 eif5a is an Important Neuroplasticity Regulator

eIF5A1 binds to the ribosome that regulates mRNA translation. In previous studies, we have found that eIF5A1 could increases neuromuscular junctions [24]. Here, LPS treatment showed an increased expression of eIF5A1 in NAc neurons (Fig. 1). We further knocked down eIF5A1 in NAc neurons, and the results showed that the expression of eIF5A1 was reduced to the level of the control group, and the spine density and depression symptoms was also reduced to the control level (Fig. 3). Since shRNA is used as a tool for knocking down genes, its own characteristics make it impossible to further reduce eIF5A1 expression. A better tool is knockout mice, but homozygous eIF5A1 embryos died early in embryonic development [40]. Therefore, we will try to create conditional knockout mice in the future. After proteomic experiments, the results showed that eIF5A1 regulated neuroplasticity-associated proteins (Fig. 5). Among them, Rab GDIα (GDIA) regulates the GDP/GTP exchange reaction of most Rab proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them. It's thought to function in the processes by which transport vesicles identify and/or fuse with their respective target membranes [41]. Lack of αGDI impairs synaptic vesicle biogenesis and recycling in the hippocampus [42]. SNAP-25(SNP25) and VAMP-2 are major SNARE protein of synaptic vesicles which mediates fusion of synaptic vesicles to release neurotransmitters [43]. And we also demonstrated that the intermediate filament protein vimentin increased neurite outgrowth [44]. Thus eIF5A1 promotes neuroplasticity by increasing the translation of these proteins.

4.4 The Mechanism of Ribosomal eIF5A1 Action needs Further Exploration

Human eIF5A1 consists of 154 amino acids and is highly conserved in eukaryotic cells [24]. eIF5A1 is the only protein found to contain the uncommon amino acid, hypusine. The 50th lysine residue of eIF5A1 is catalyzed by deoxyhypusine synthase to form deoxyhypusine, which is then hydroxylated by deoxyhypusine hydroxylase to form hypusine [45]. eIF5A1 stimulates the translation of mRNA. Knockdown of eIF5A1 results in reduced protein synthesis and polysome size. Conversely, adding purified eIF5A1 to *in vitro* cells can significantly restore protein synthesis and rescue the cells from eIF5A1 inhibition [46].

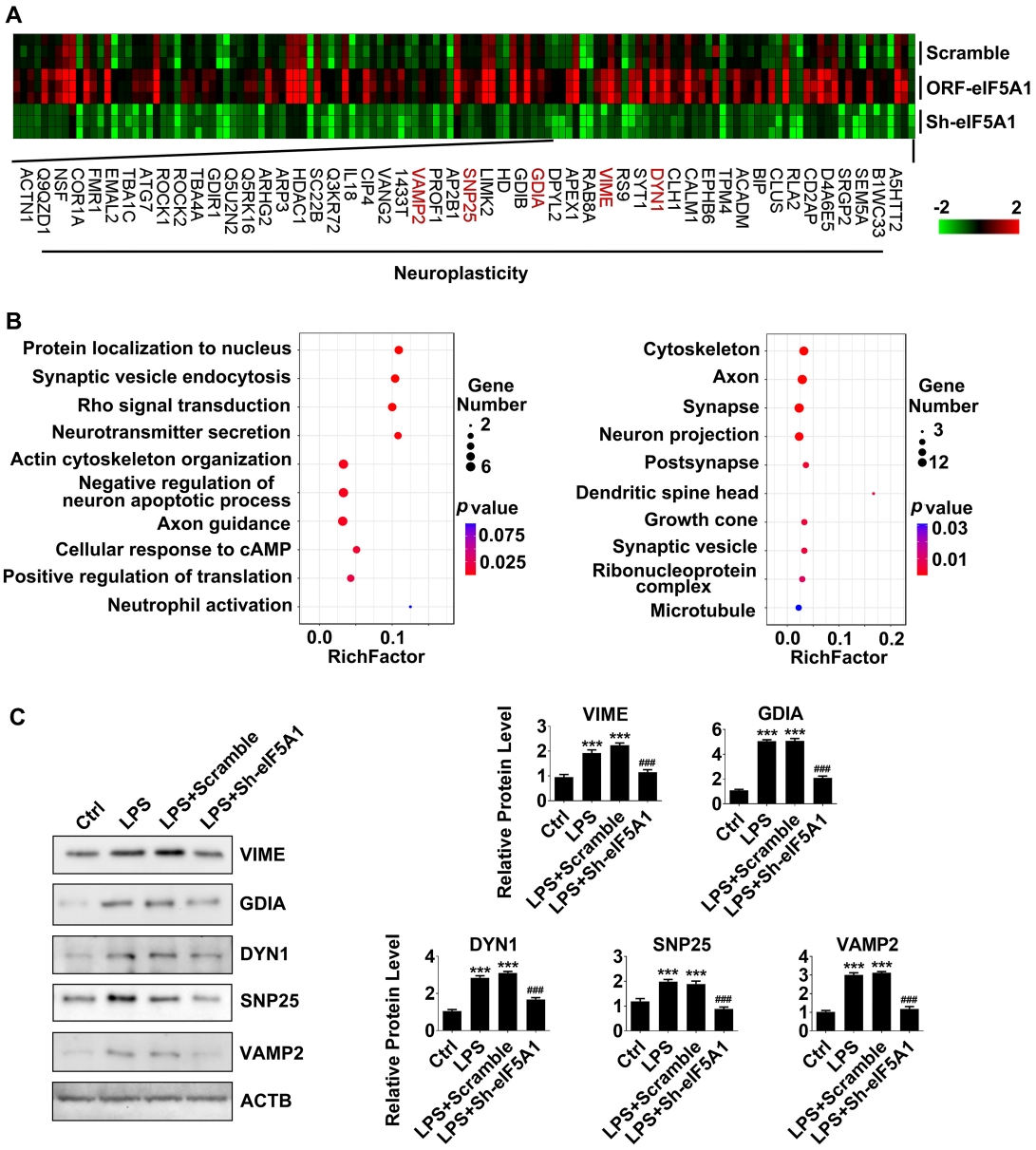

Fig. 5. Proteome analysis showed that eIF5A1 regulates neuroplasticity-related proteins. (A) eIF5A1 expression was regulated in cultured NAc neurons. The heatmap of proteomics results showed differentially expressed proteins (>2 fold-change, $p < 0.05$). (B) Functional cluster analysis of differentially expressed proteins. (C) Western blotting analysis showed that eIF5A1 knockdown significantly decreased neuroplasticity-associated proteins in rats' NAc. n = 8 per group. *** $p < 0.001$, compared with Ctrl group; ### $p < 0.001$, compared with LPS + Scramble group.

Initially, eIF5A1 and its homologous protein EF-P were thought to play a role in the initiation of protein translation. It was speculated that eIF5A1 is not involved in the entire process of protein synthesis, but only in the formation of the first peptide bond [47,48]. However, this molecule was also subsequently found to contribute to the elongation process during protein translation [46,49]. There is now evidence that eIF5A1 stimulates elongation, but not initiation. Reduction of the eIF5A1 level in yeast was observed to decrease protein synthesis by only 30%, raising doubts about eIF5A1 as a translation initiation factor [50]. More recent studies have re-examined the role of eIF5A1 in protein translation. It has been demonstrated that eIF5A1 binds to active ribosomes, suggesting it plays a central role in the control of translation [51]. Another important finding was that eIF5A1 accelerates the translation of proteins containing polyproline structures (PPP or PPG), thus preventing the stall of translational due to polyproline [20,21]. Cryo-precipitation electron microscopy has revealed that eIF5A1 binds to ribosomal 80S and is localized between the P and E sites. The hypusine residue of eIF5A1 can be inserted into the pocket formed by the CAA end of P-tRNA. In this

way, eIF5A1 assists the translation of polyproline by stabilizing the ribosomal structure, rather than by catalyzing the synthesis of peptide bonds [52]. eIF5A1 can also interact with eIF2 by binding eIF2-GTP-Met-tRNAi^{Met} and thereby stimulating its binding to the 40S ribosomal subunit, causing eIF2 to dissociate from the ribosome. Ultimately, inhibition of GDP dissociation by eIF5A1 promotes translation of the protein [53]. Taken together, these results have clarified the role of eIF5A1 in protein translation. However, the central role of eIF5A1 in translation still requires further exploration.

5. Conclusions

In the present study we investigated whether eIF5A1 was elevated in the NAc of LPS-treated rats and whether it regulated neuroplasticity. Our results indicate that increased neuroplasticity of NAc may be due to up-regulation of eIF5A1 by LPS. This finding provides new evidence that the brain's reward system is involved in depression.

Author Contributions

QL and FFS designed the research study. QL, HZ and LL performed the research. WL, XH and FFS analyzed the data. QL and FFS wrote the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animal protocols were approved by The Ethics Committee of Chongqing Medical University (No. 2019-0013).

Acknowledgment

We are very grateful to the reviewers for excellent comments of the article.

Funding

This work was supported by the Science & Technology Department of Sichuan Province, No. 2021YJ0174 (to QL); and National Natural Science Foundation of China, No. 82001269 (to FFS); No. 81860234 (to XH); and Scientific and Technological Research Program of Chongqing Municipal Education Commission, No. KJQN202100456 (to FFS). The funding bodies played no role in the study design, in the collection, analysis and interpretation of data, in the writing of the paper, and in the decision to submit the paper for publication.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2201014.

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